IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: SDS-PAGE ISOLATION OF PEPTIDES

(CNBr CLEAVAGE OF UNABSORBED TMAE FRACTION FOLLOWED BY SDS-PAGE AND N-TERM SEQUENCING OF FRAGMENTS FROM PVDF BLOT)

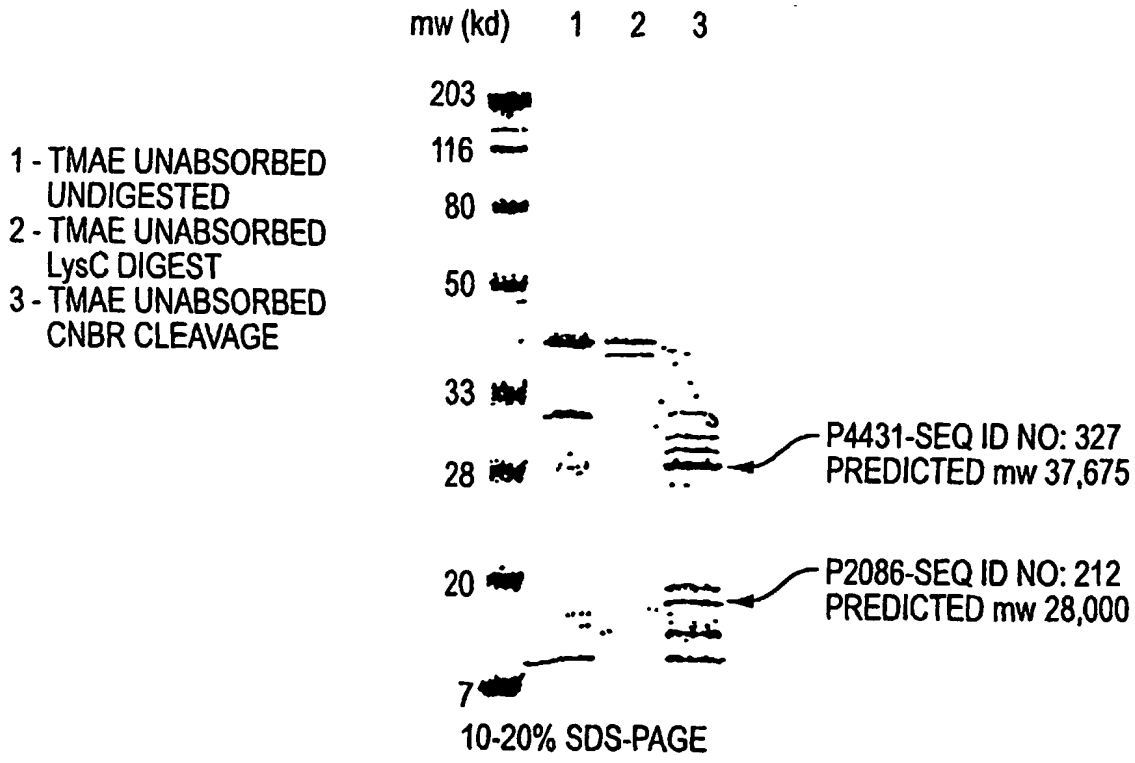


FIG. 1A

IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: REVERSE PHASE ISOLATION OF PEPTIDES

ENZYMATIC DIGESTION OF UNABSORBED TMAE FRACTION FOLLOWED BY REVERSE PHASE CHROMATOGRAPHY SEPARATION OF PEPTIDES AND DIRECT N-TERMINAL SEQUENCING

ENZYMATIC DIGEST	RETENTION TIME OF PEPTIDE (min)	MOLECULAR WEIGHT OF PEPTIDE (d)	N-TERM. ID
GluC (V8)	6.716	2069.7	P5163
LysC	13.800	3351.2	P4431
LysC	13.800	3351.2	P2086
ArgC	6.860	2278.9	P5163

P4431 (SEQ ID NO: 327)
PREDICTED mw 36,775

P2086 (SEQ ID NO: 212)
PREDICTED mw 27,100

P5163 (SEQ ID NO: 328)
PREDICTED mw 7,081

FIG. 1B

PURIFICATION OF rLP2086

BLR DE3 pLys CELLS
pET27b 2086A1

↓

SARCOSYL EXTRACT

↓

TMAE CHROMATOGRAPHY

↓

SP SEPHAROSE CHROMATOGRAPHY
84% HOMOGENEITY

1 - SARCOSYL EXTRA
2 - SP POOL

10-20% SDS-PAGE
COOMASSIE STAIN

FIG. 2

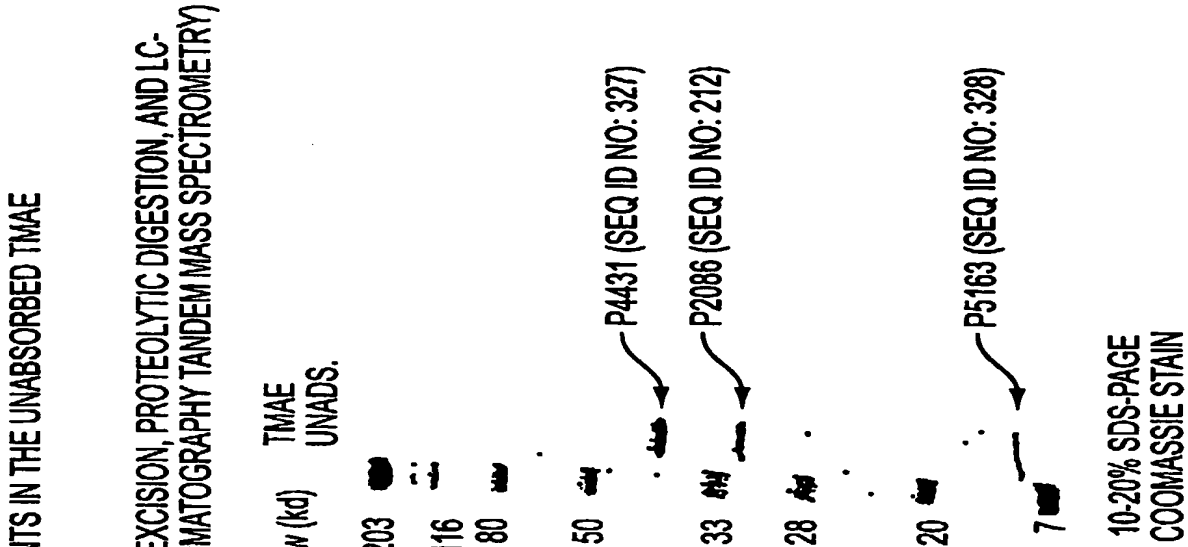

IDENTIFICATION OF COMPONENTS IN THE UNABSORBED TMAE FRACTION: LC-MS/MS
SDS-PAGE FOLLOWED BY GEL EXCISION, PROTEOLYTIC DIGESTION, AND LC-MS/MS ANALYSIS (LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY)
mw (kd)
TMAE UNADS.
203
116
80
50
33
28
20
7
P4431 (SEQ ID NO: 327)
P2086 (SEQ ID NO: 212)
P5163 (SEQ ID NO: 328)
10-20% SDS-PAGE COOMASSIE STAIN

FIG. 3

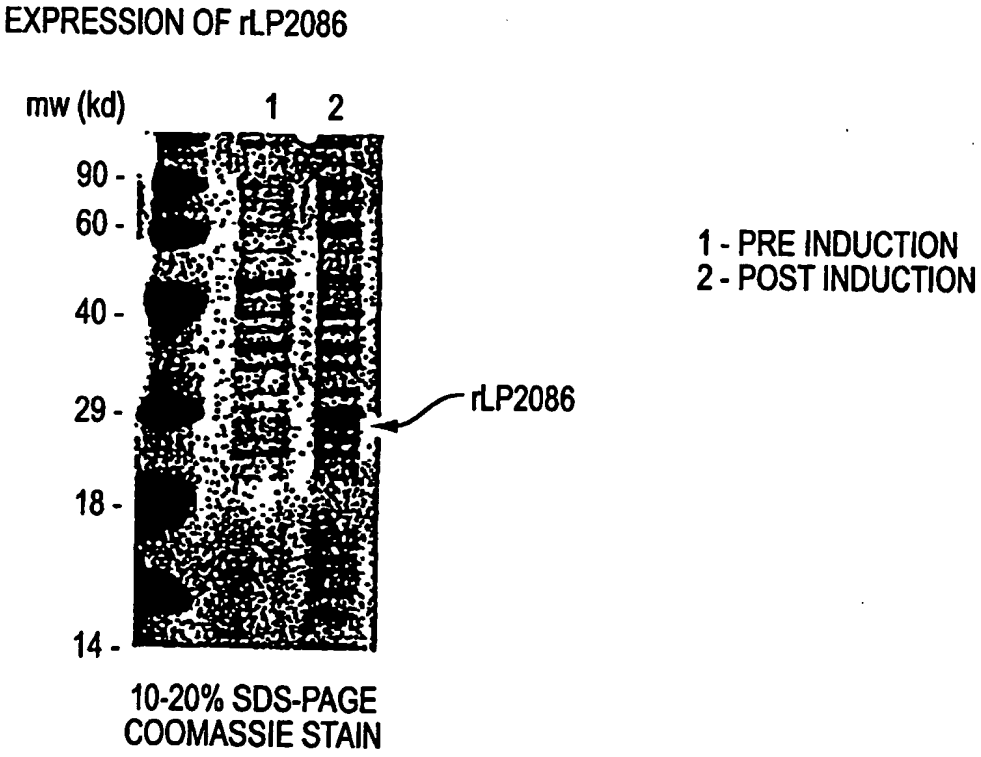

EXPRESSION OF rLP2086
mw (kd)
1
2
90 -
60 -
40 -
29 -
18 -
14 -
rLP2086
1 - PRE INDUCTION
2 - POST INDUCTION
10-20% SDS-PAGE
COOMASSIE STAIN

FIG. 4

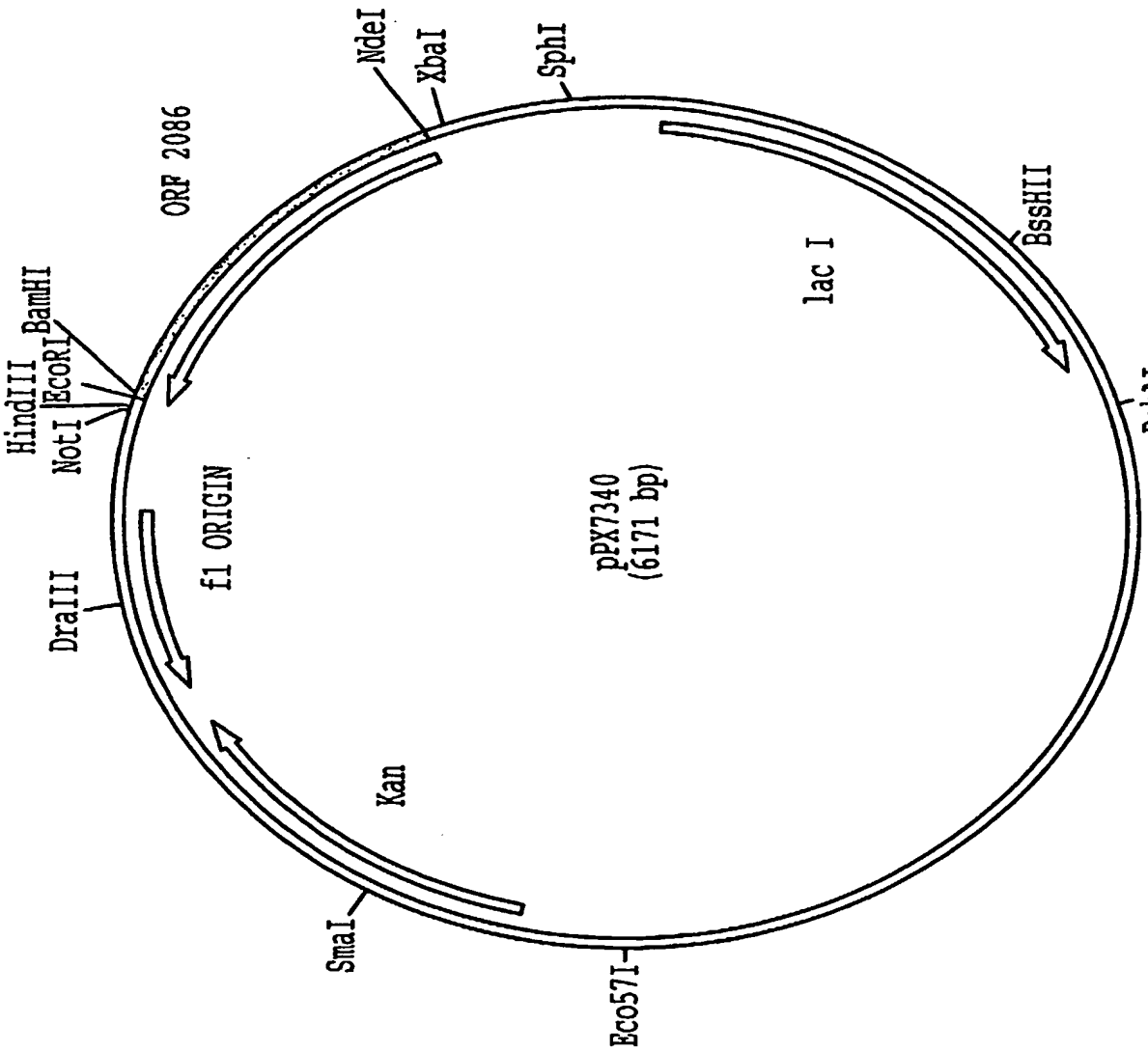

NdeI
XbaI
SphI
ORF 2086
BssHII
lac I
BamHI
EcoRI
HindIII
NotI
PshAI
pPX7340
(6171 bp)
f1 ORIGIN
DraIII
Kan
SmaI
Eco57I

FIG. 5

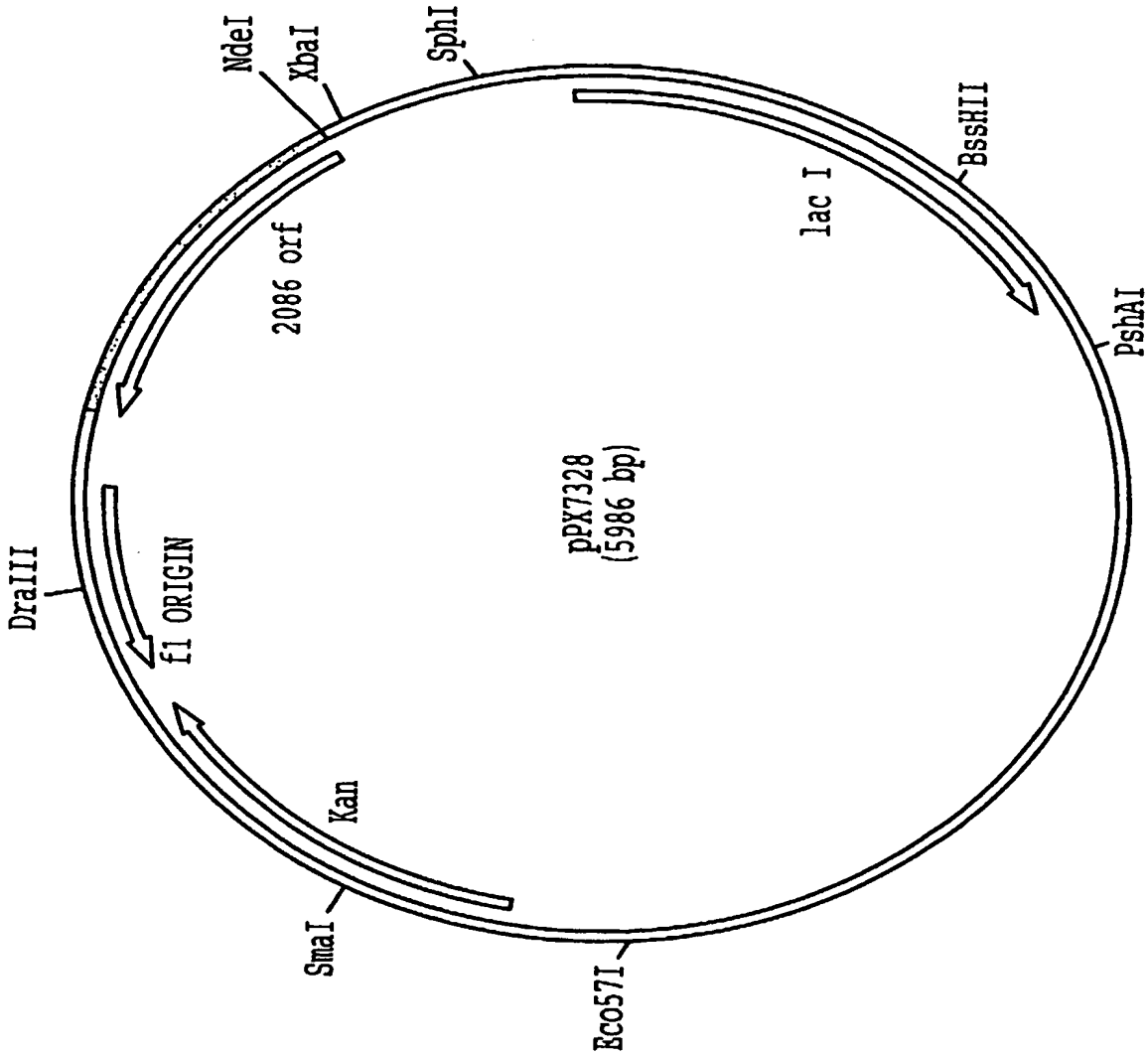

NdeI
XbaI
SphI
BssHII
PshAI
Eco57I
SmaI
Kan
DraIII
f1 ORIGIN
2086 orf
lac I
pPX7328
(5986 bp)

FIG. 6

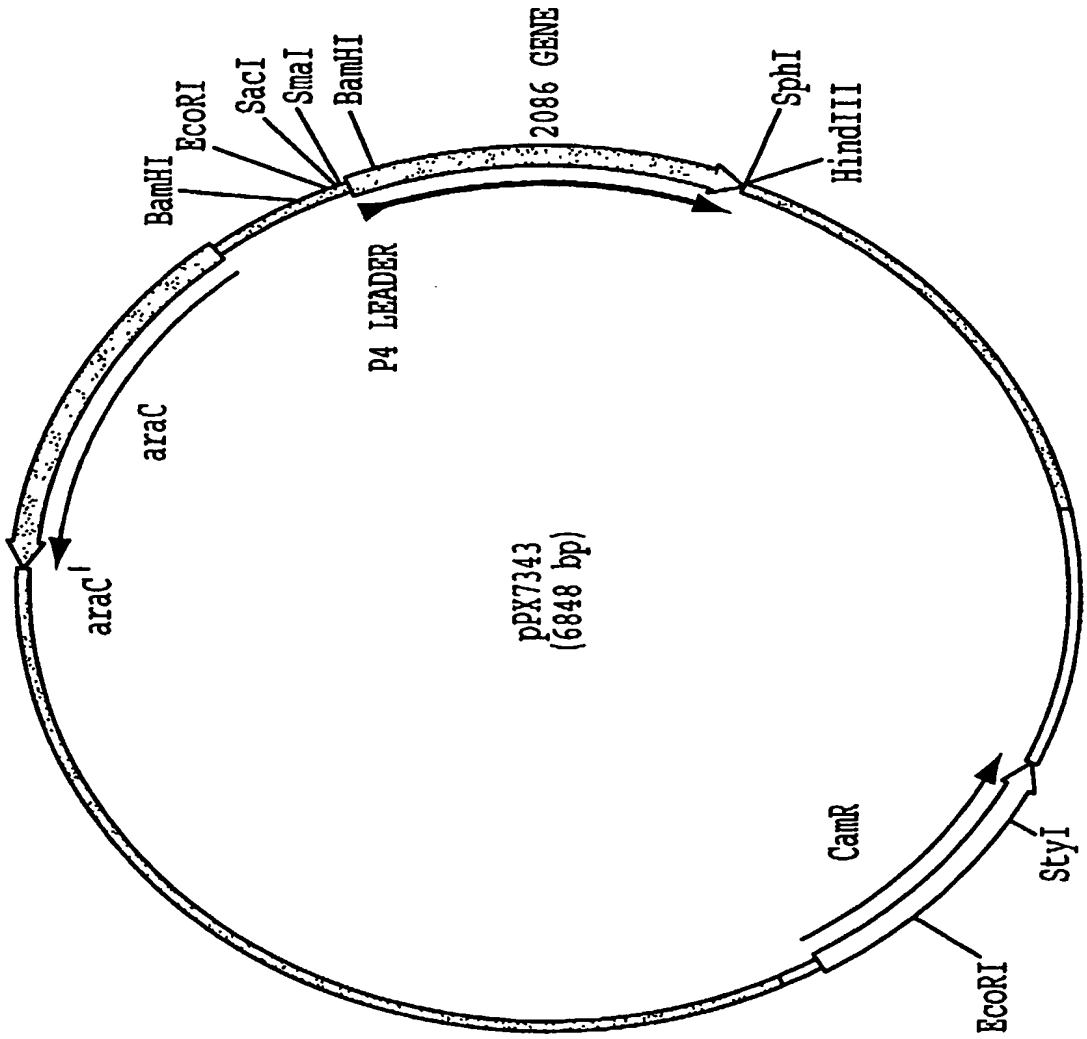

BamHI
EcoRI
SacI
SmaI
BamHI
2086 GENE
SphI
HindIII
P4 LEADER
araC
araC'
pPX7343
(6848 bp)
CamR
StyI
EcoRI

FIG. 7

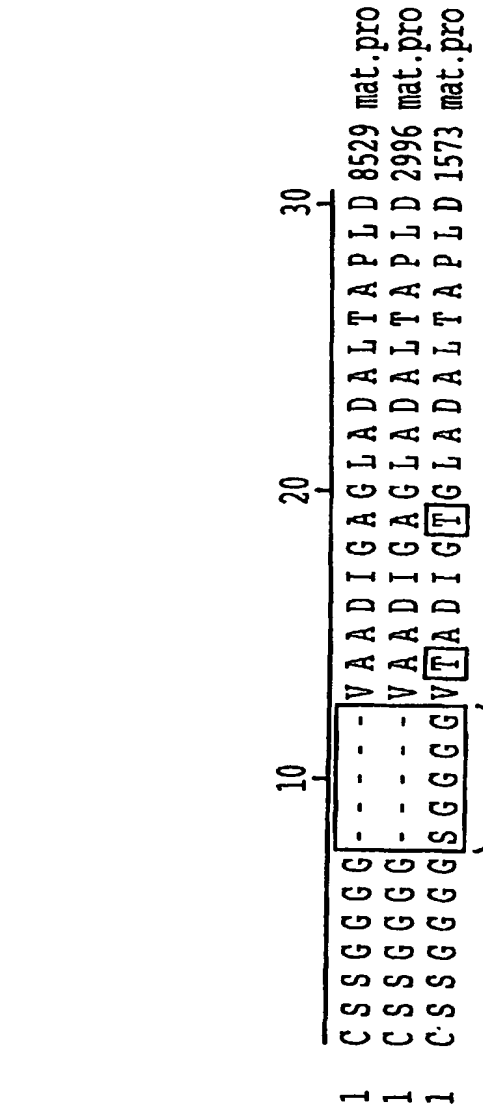

1 C S S G G G G G - - - - - V A A D I G A G L A D A L T A P L D 8529 mat.pro
1 C S S G G G G G - - - - - V A A D I G A G L A D A L T A P L D 2996 mat.pro
1 C S S G G G G G S G G G G V T A D I G T G L A D A L T A P L D 1573 mat.pro
10
20
30
5 AMINO ACID REPEAT

FIG. 8

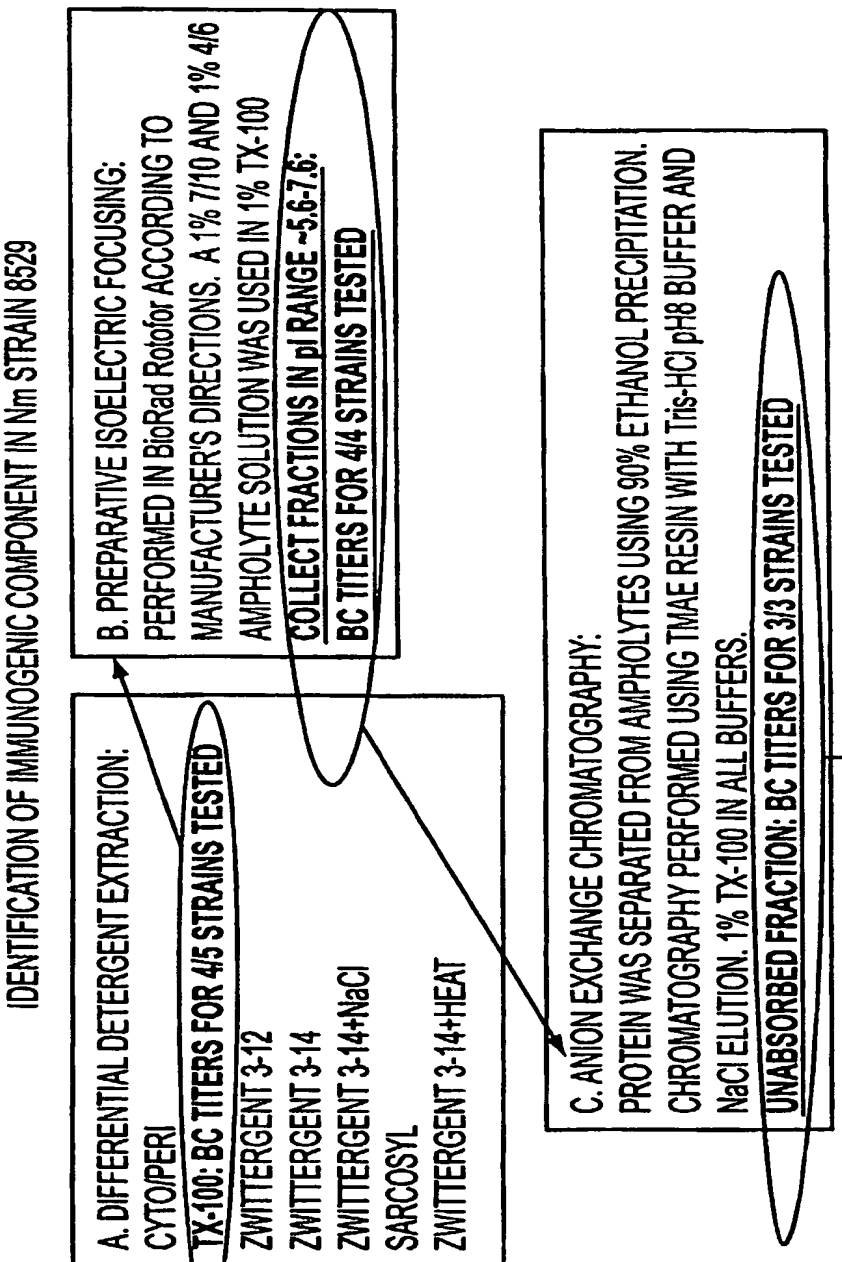

IDENTIFICATION OF IMMUNOGENIC COMPONENT IN Nm STRAIN 8529
A. DIFFERENTIAL DETERGENT EXTRACTION:
CYTO/PERI
TX-100: BC TITERS FOR 4/5 STRAINS TESTED
ZWITTERGENT 3-12
ZWITTERGENT 3-14
ZWITTERGENT 3-14+NaCl
SARCOSYL
ZWITTERGENT 3-14+HEAT
B. PREPARATIVE ISOELECTRIC FOCUSING:
PERFORMED IN BioRad Rotofor ACCORDING TO
MANUFACTURER'S DIRECTIONS. A 1% 7/10 AND 1% 4/6
AMPHOLYTE SOLUTION WAS USED IN 1% TX-100
COLLECT FRACTIONS IN pI RANGE ~5.6-7.6:
BC TITERS FOR 4/4 STRAINS TESTED
C. ANION EXCHANGE CHROMATOGRAPHY:
PROTEIN WAS SEPARATED FROM AMPHOLYTES USING 90% ETHANOL PRECIPITATION.
CHROMATOGRAPHY PERFORMED USING TMAE RESIN WITH Tris-HCl pH8 BUFFER AND
NaCl ELUTION. 1% TX-100 IN ALL BUFFERS.
UNABSORBED FRACTION: BC TITERS FOR 3/3 STRAINS TESTED
TO FIG. 9B

FIG. 9A

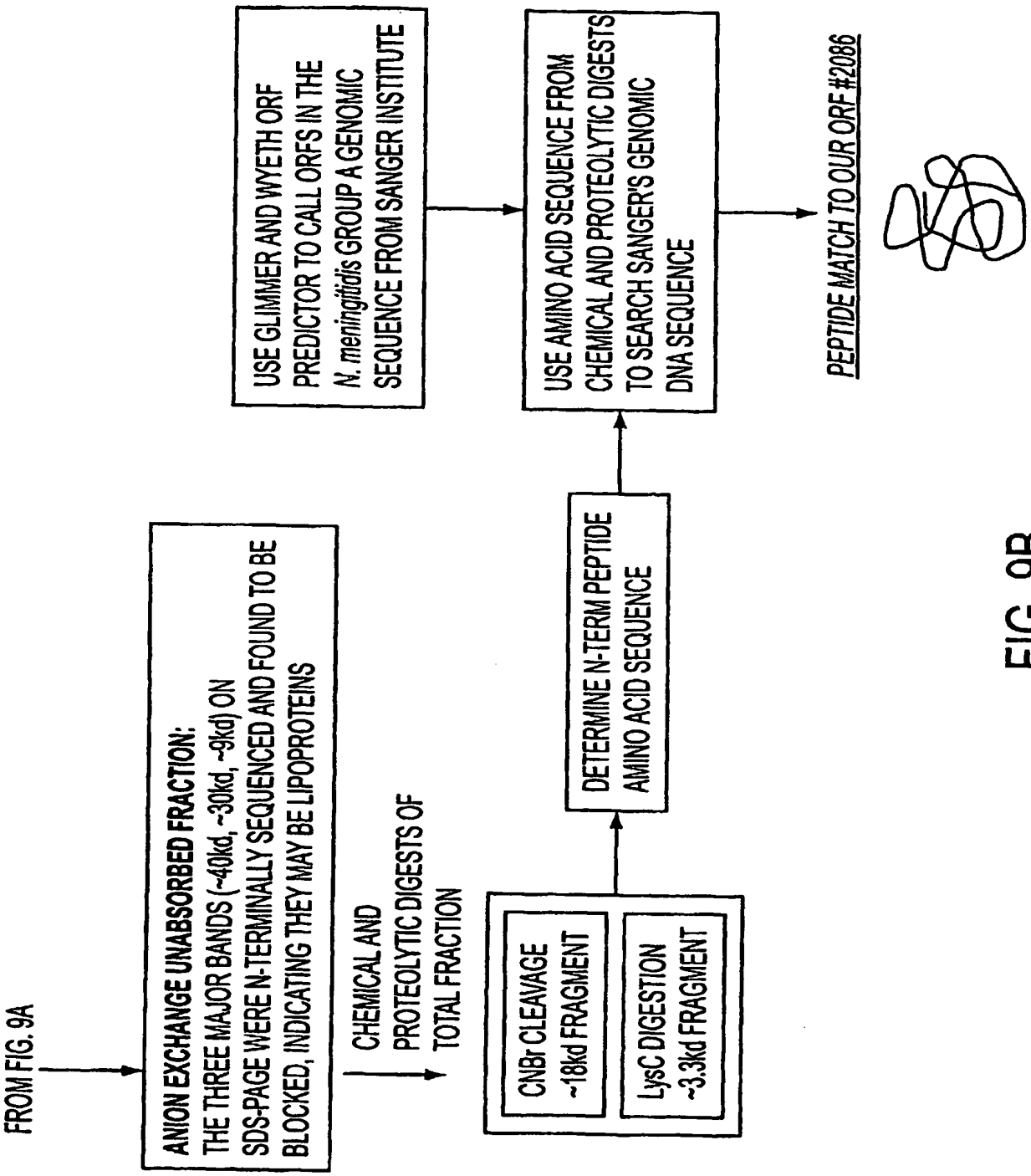

FROM FIG. 9A
ANION EXCHANGE UNABSORBED FRACTION:
THE THREE MAJOR BANDS (~40kd, ~30kd, ~9kd) ON SDS-PAGE WERE N-TERMINALLY SEQUENCED AND FOUND TO BE BLOCKED, INDICATING THEY MAY BE LIPOPROTEINS
CHEMICAL AND PROTEOLYTIC DIGESTS OF TOTAL FRACTION
CNBr CLEAVAGE ~18kd FRAGMENT
LysC DIGESTION ~3.3kd FRAGMENT
DETERMINE N-TERM PEPTIDE AMINO ACID SEQUENCE
USE GLIMMER AND WYETH ORF PREDICTOR TO CALL ORFS IN THE N. meningitidis GROUP A GENOMIC SEQUENCE FROM SANGER INSTITUTE
USE AMINO ACID SEQUENCE FROM CHEMICAL AND PROTEOLYTIC DIGESTS TO SEARCH SANGER'S GENOMIC DNA SEQUENCE
PEPTIDE MATCH TO OUR ORF #2086

FIG. 9B

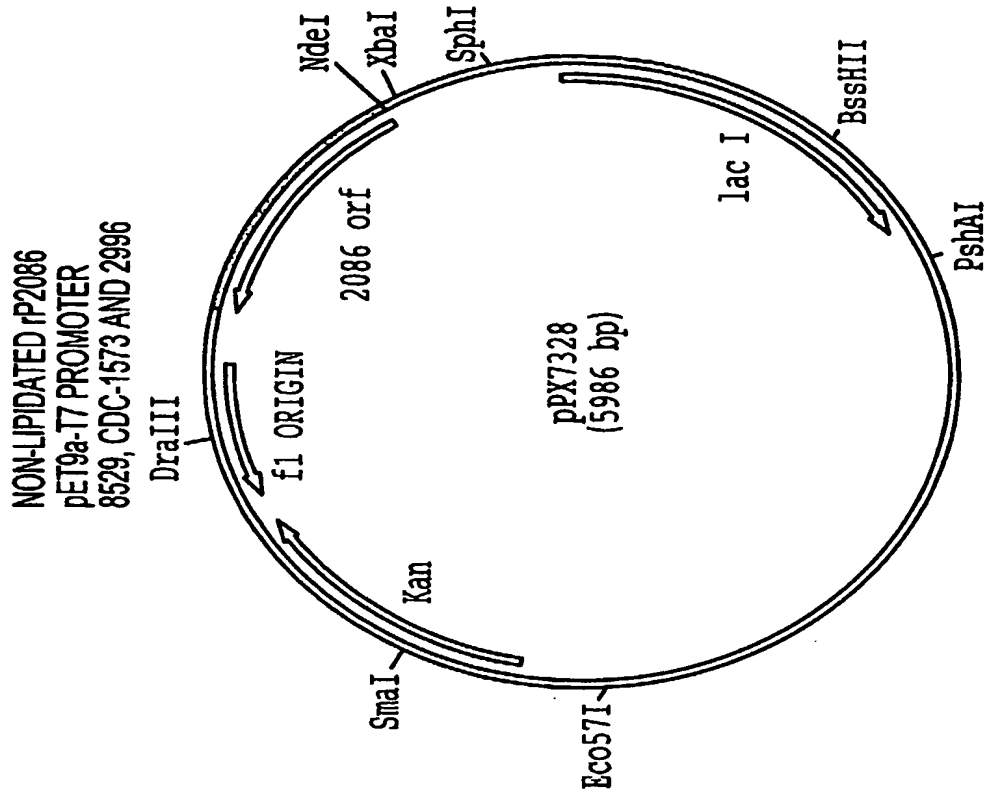


FIG. 10B

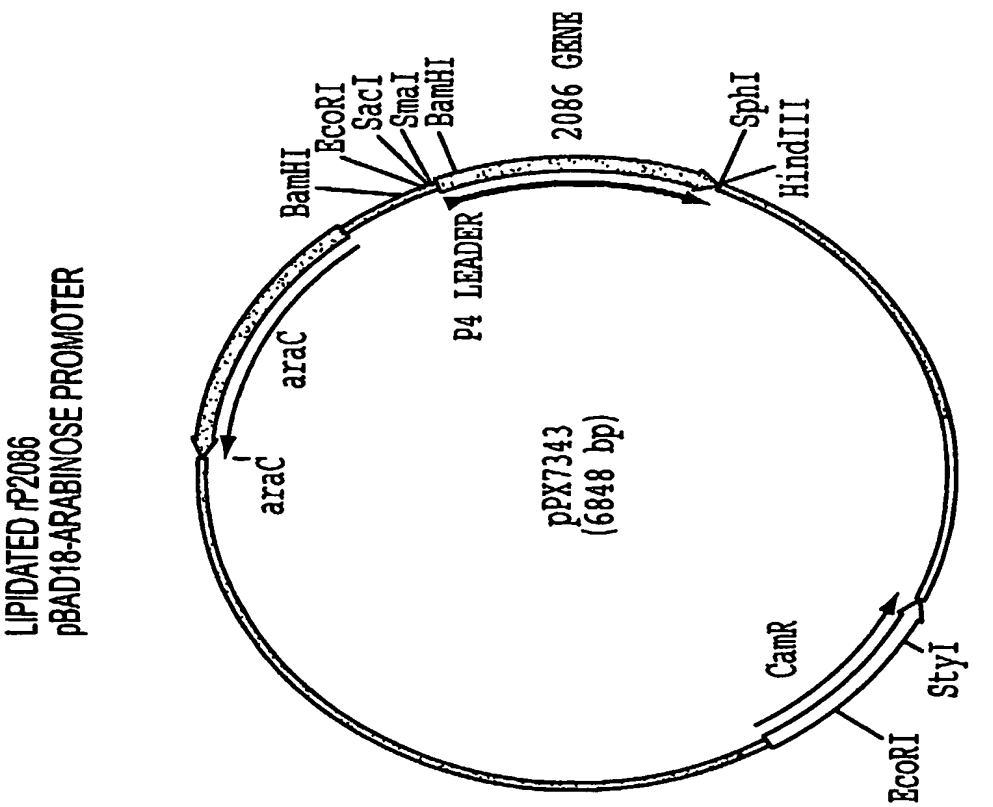


FIG.10A

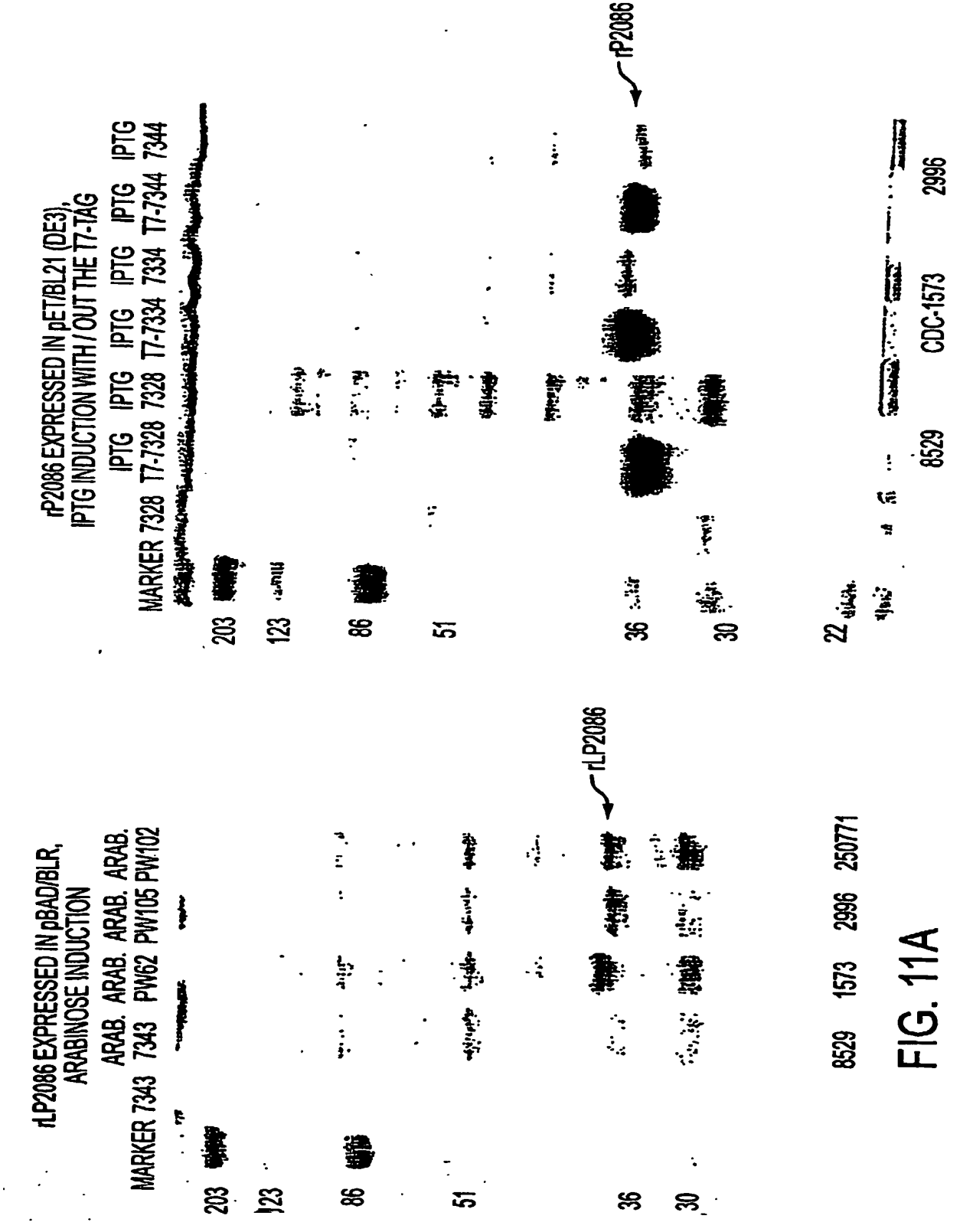


FIG. 11A

FIG. 11B

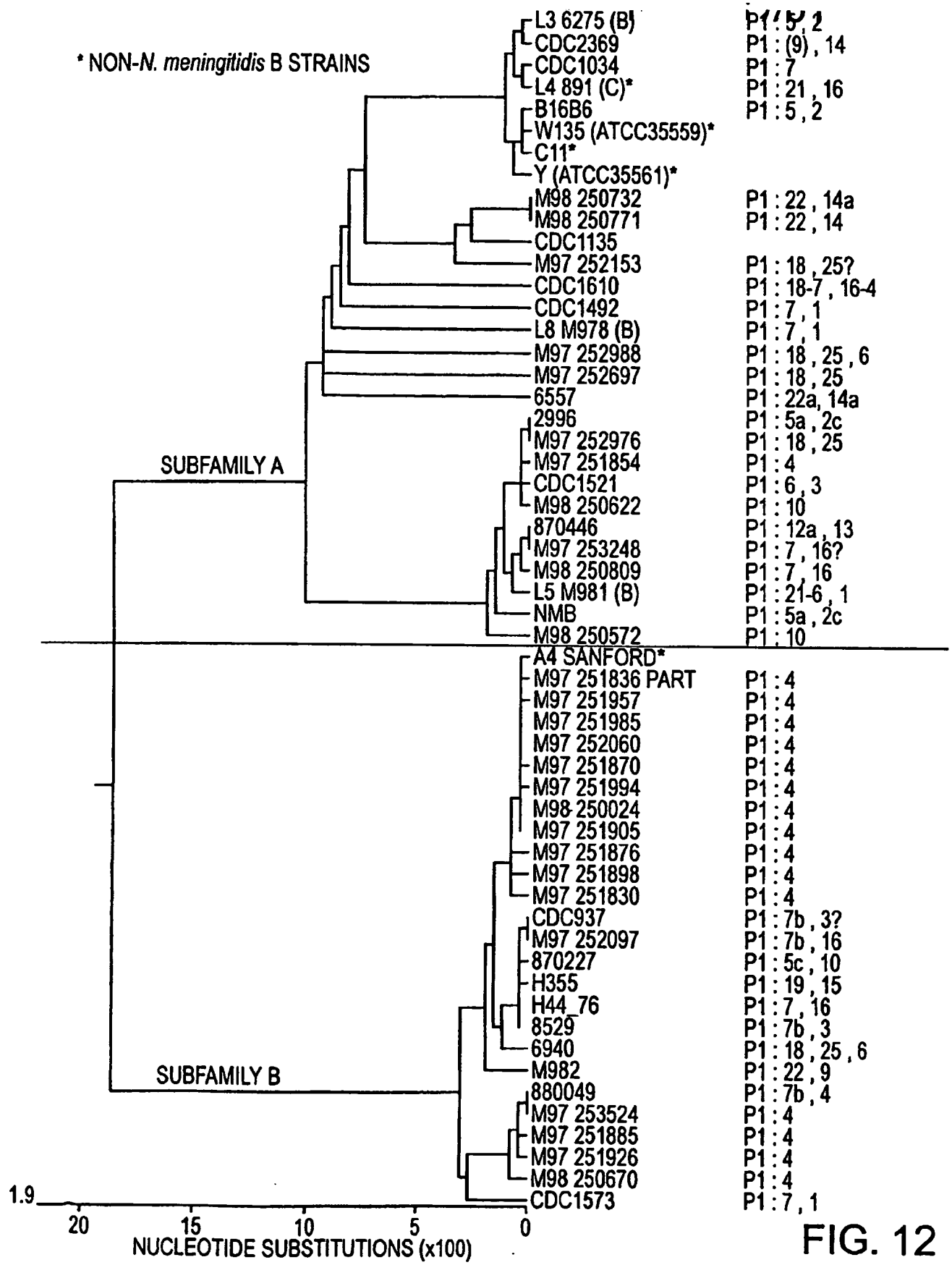

* NON-N. meningitidis B STRAINS
SUBFAMILY A
SUBFAMILY B
L3 6275 (B) P1 : 5 , 2
CDC2369 P1 : (9) , 14
CDC1034 P1 : 7
L4 891 (C)* P1 : 21 , 16
B16B6 P1 : 5 , 2
W135 (ATCC35559)*
C11*
Y (ATCC35561)*
M98 250732 P1 : 22 , 14a
M98 250771 P1 : 22 , 14
CDC1135
M97 252153 P1 : 18 , 25?
CDC1610 P1 : 18-7 , 16-4
CDC1492 P1 : 7 , 1
L8 M978 (B) P1 : 7 , 1
M97 252988 P1 : 18 , 25 , 6
M97 252697 P1 : 18 , 25
6557 P1 : 22a, 14a
2996 P1 : 5a , 2c
M97 252976 P1 : 18 , 25
M97 251854 P1 : 4
CDC1521 P1 : 6 , 3
M98 250622 P1 : 10
870446 P1 : 12a , 13
M97 253248 P1 : 7 , 16?
M98 250809 P1 : 7 , 16
L5 M981 (B) P1 : 21-6 , 1
NMB P1 : 5a , 2c
M98 250572 P1 : 10
A4 SANFORD*
M97 251836 PART P1 : 4
M97 251957 P1 : 4
M97 251985 P1 : 4
M97 252060 P1 : 4
M97 251870 P1 : 4
M97 251994 P1 : 4
M98 250024 P1 : 4
M97 251905 P1 : 4
M97 251876 P1 : 4
M97 251898 P1 : 4
M97 251830 P1 : 4
CDC937 P1 : 7b , 3?
M97 252097 P1 : 7b , 16
870227 P1 : 5c , 10
H355 P1 : 19 , 15
H44_76 P1 : 7 , 16
8529 P1 : 7b , 3
6940 P1 : 18 , 25 , 6
M982 P1 : 22 , 9
880049 P1 : 7b , 4
M97 253524 P1 : 4
M97 251885 P1 : 4
M97 251926 P1 : 4
M98 250670 P1 : 4
CDC1573 P1 : 7 , 1
1.9
20 15 10 5 0
NUCLEOTIDE SUBSTITUTIONS (x100)

FIG. 12

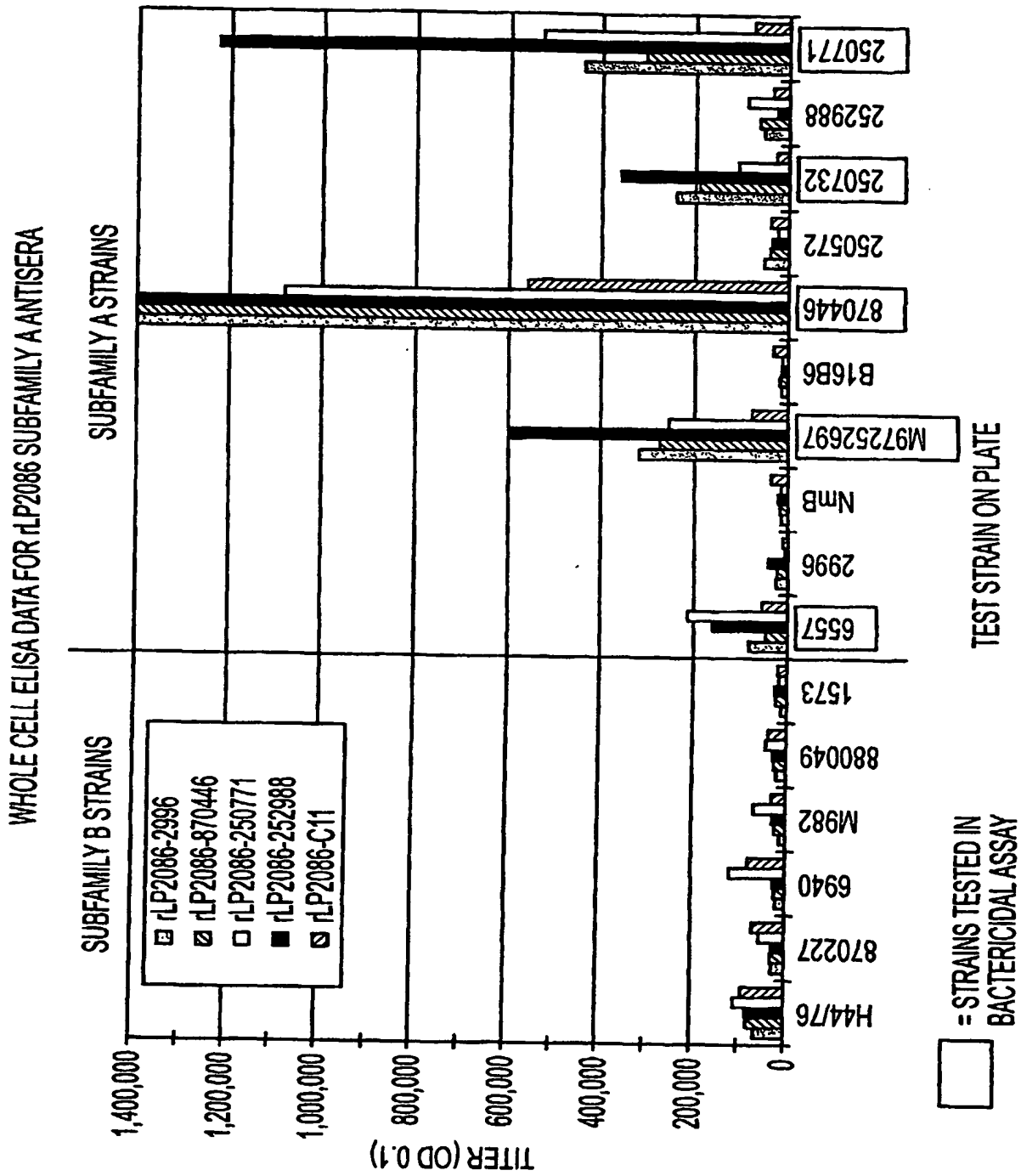

WHOLE CELL ELISA DATA FOR rLP2086 SUBFAMILY A ANTISERA
SUBFAMILY A STRAINS
SUBFAMILY B STRAINS
rLP2086-2996
rLP2086-870446
rLP2086-250771
rLP2086-252988
rLP2086-C11
TITER (OD 0.1)
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
H44/76
870227
6940
M982
880049
1573
6557
2996
NmB
M97252697
B16B6
870446
250572
250732
252988
250771
TEST STRAIN ON PLATE
= STRAINS TESTED IN BACTERICIDAL ASSAY

FIG. 13

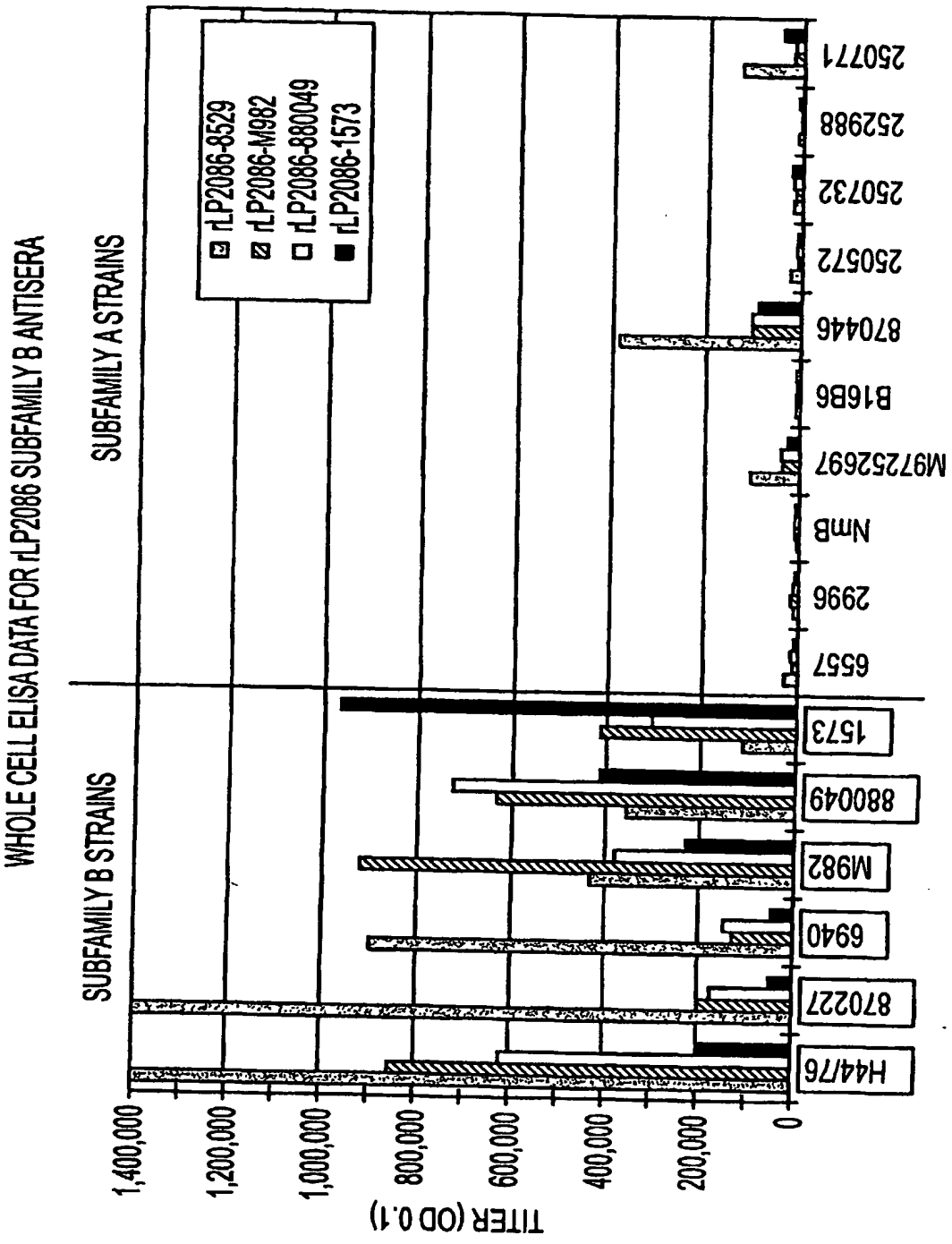
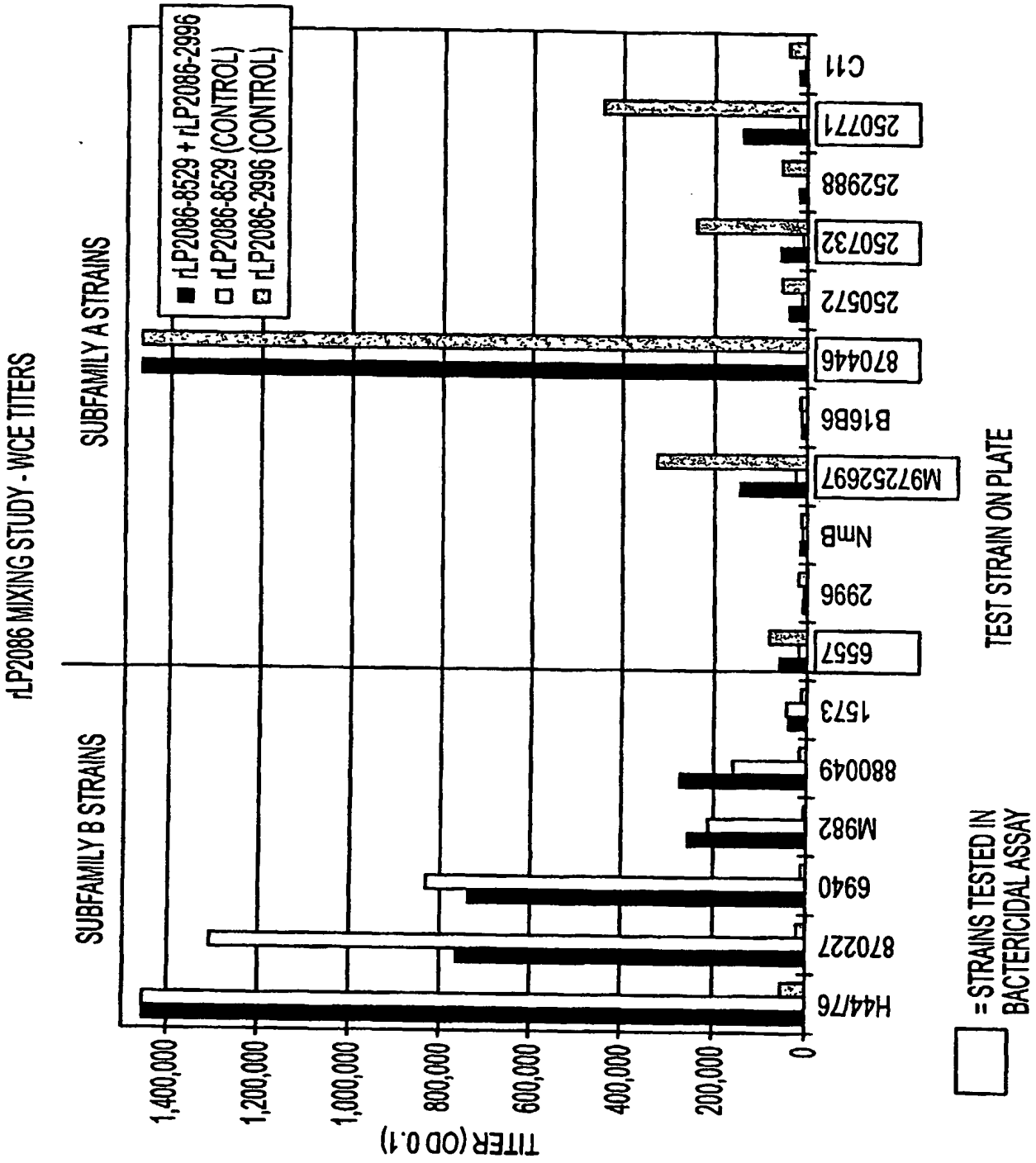
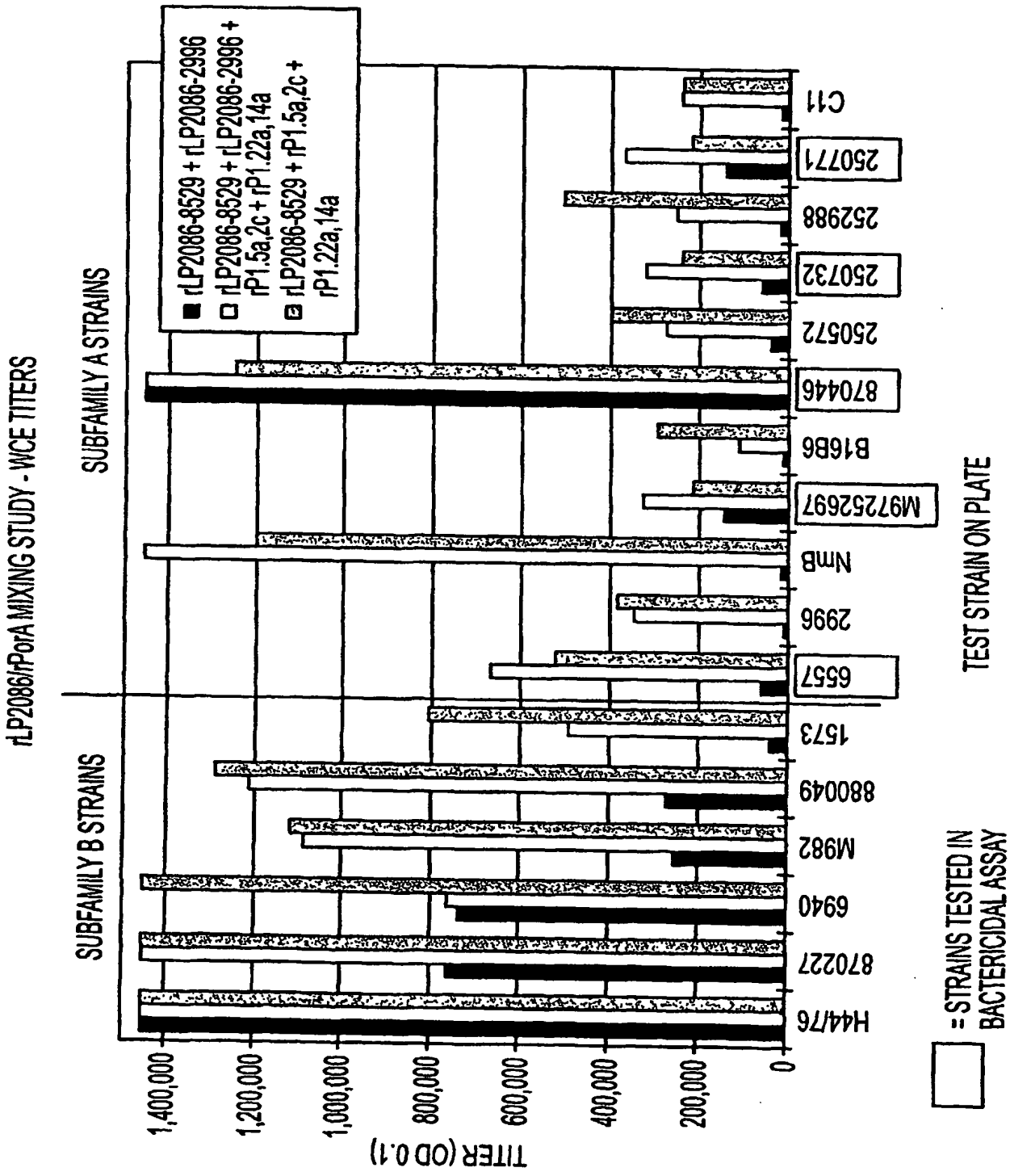
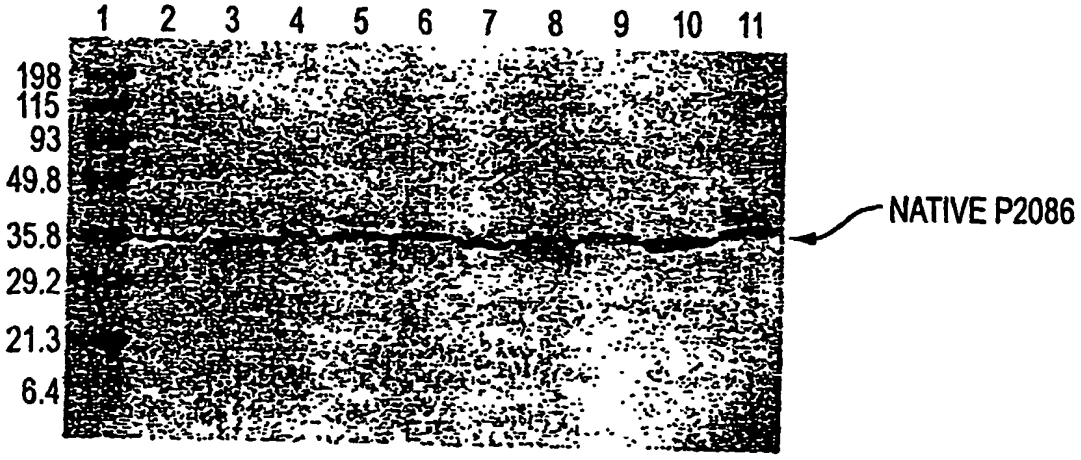

WHOLE CELL ELISA DATA FOR rLP2086 SUBFAMILY B ANTISERA
SUBFAMILY A STRAINS
SUBFAMILY B STRAINS
rLP2086-8529
rLP2086-M982
rLP2086-880049
rLP2086-1573
TITER (OD 0.1)
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
H44/76
870227
6940
M982
880049
1573
6557
2996
NmB
M97252697
B16B6
870446
250572
250732
252988
250771
TEST STRAIN ON PLATE
= STRAINS TESTED IN BACTERICIDAL ASSAY

FIG 14


rLP2086 MIXING STUDY - WCE TITERS
rLP2086-8529 + rLP2086-2996
rLP2086-8529 (CONTROL)
rLP2086-2996 (CONTROL)
SUBFAMILY A STRAINS
SUBFAMILY B STRAINS
TITER (OD 0.1)
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
H44/76
870227
6940
M982
880049
1573
6557
2996
NmB
M97252697
B16B6
870446
250572
250732
252988
250771
C11
TEST STRAIN ON PLATE
= STRAINS TESTED IN BACTERICIDAL ASSAY


rLP2086/rPorA MIXING STUDY - WCE TITERS
SUBFAMILY A STRAINS
SUBFAMILY B STRAINS
rLP2086-8529 + rLP2086-2996
rLP2086-8529 + rLP2086-2996 + rP1.5a,2c + rP1.22a,14a
rLP2086-8529 + rP1.5a,2c + rP1.22a,14a
TITER (OD 0.1)
1,400,000
1,200,000
1,000,000
800,000
600,000
400,000
200,000
0
H44/76
870227
6940
M982
880049
1573
6557
2996
NmB
M97252697
B16B6
870446
250572
250732
252988
250771
C11
TEST STRAIN ON PLATE
= STRAINS TESTED IN BACTERICIDAL ASSAY

WESTERN BLOT REACTIVITY OF rLP2086 MOUSE ANTISERA TO P2086 SUBFAMILY B *N. meningitidis* WHOLE CELL LYSATES

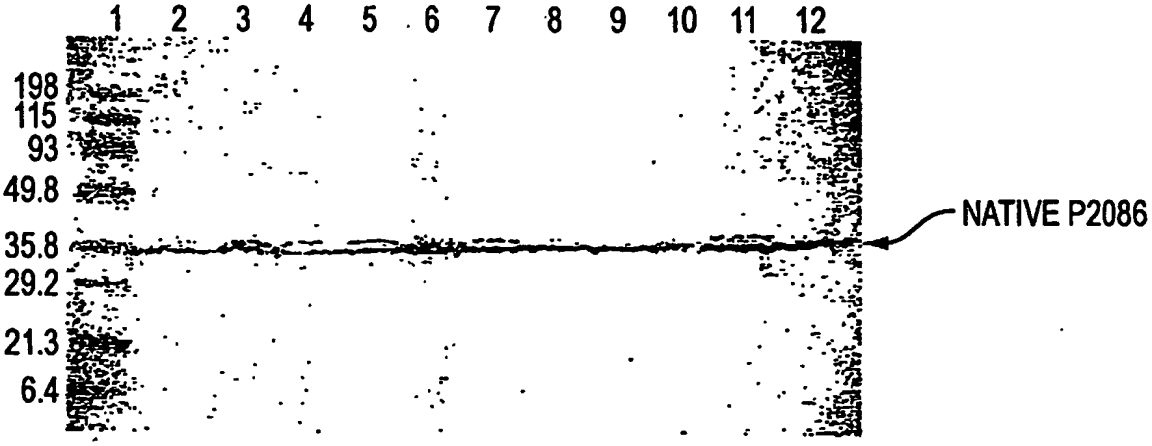


1 - MOLECULAR WEIGHT MARKER (kDa)
2 - M97 251985
3 - CDC937
4 - 6940
5 - M97 251926
6 - CDC1573
7 - CDC1359
8 - CDC1658
9 - M97 252026
10 - M97 252029
11 - M982

SUBFAMILY B P2086 CELL LYSATES ARE ALL GROUP B *N. meningitidis*

FIG. 17

WESTERN BLOT REACTIVITY OF rLP2086 MOUSE ANTISERA TO P2086 SUBFAMILY A *N. meningitidis* AND *N. lactamica* WHOLE CELL LYSATES



1 - MOLECULAR WEIGHT MARKERS (kDa)
2 - GROUP A *N. meningitidis* A4 (P2086 SUBFAMILY B)
3 - GROUP C *N. meningitidis* - C11
4 - GROUP Y *N. meningitidis* - ATCC35561
5 - GROUP W135 *N. meningitidis* - ATCC35559
6 - *N. lactamica* - UR5

GROUP B *N. meningitidis*:
7 - CDC1034
8 - M98 250732
9 - NmB
10 - 6557
11 - CDC1521
12 - M97 252153

FIG. 18

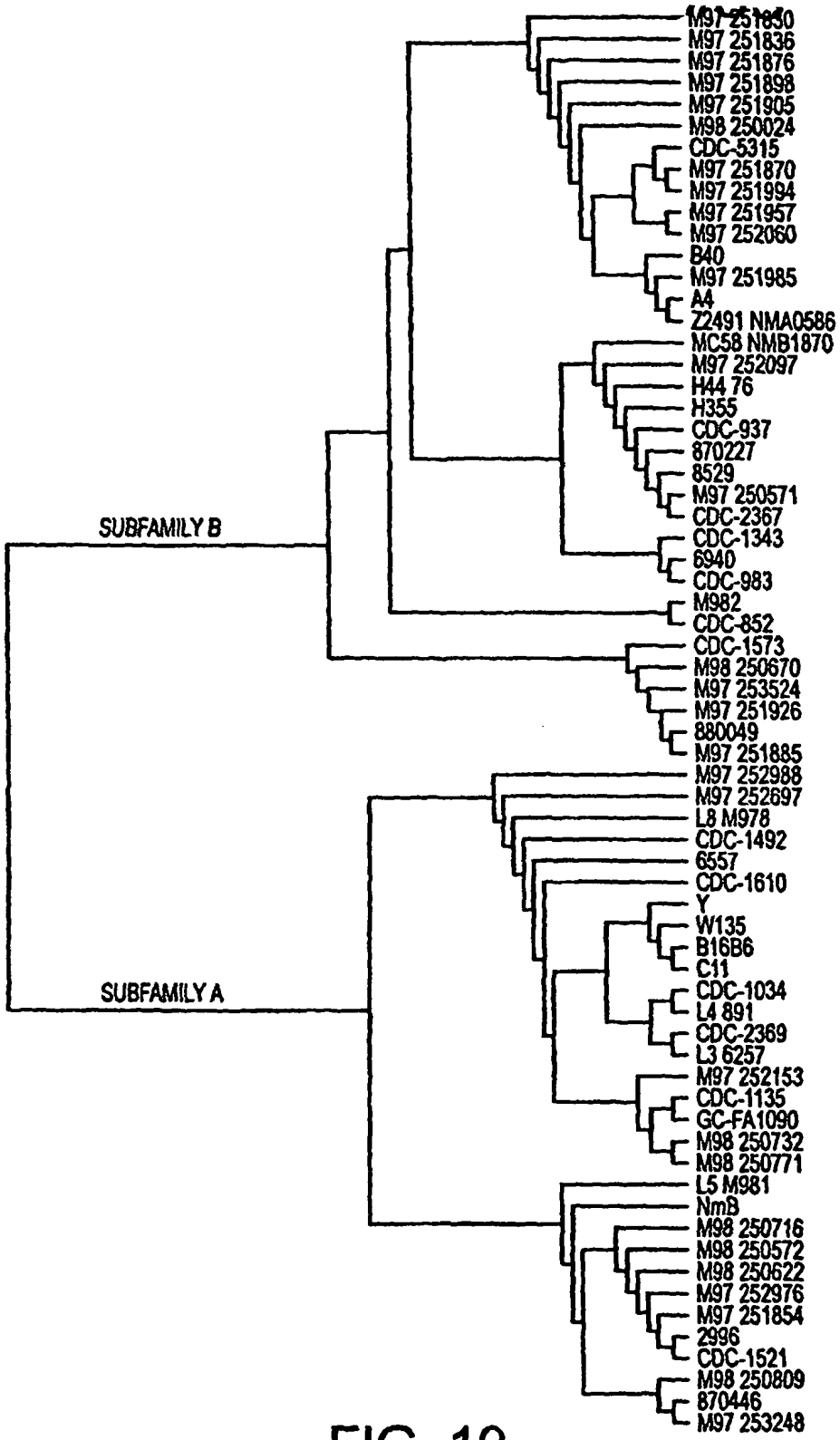

M97 251850
M97 251836
M97 251876
M97 251898
M97 251905
M98 250024
CDC-5315
M97 251870
M97 251994
M97 251957
M97 252060
B40
M97 251985
A4
Z2491 NMA0586
MC58 NMB1870
M97 252097
H44 76
H355
CDC-937
870227
8529
M97 250571
CDC-2367
CDC-1343
6940
CDC-983
M982
CDC-852
CDC-1573
M98 250670
M97 253524
M97 251926
880049
M97 251885
M97 252988
M97 252697
L8 M978
CDC-1492
6557
CDC-1610
Y
W135
B16B6
C11
CDC-1034
L4 891
CDC-2369
L3 6257
M97 252153
CDC-1135
GC-FA1090
M98 250732
M98 250771
L5 M981
NmB
M98 250716
M98 250572
M98 250622
M97 252976
M97 251854
2996
CDC-1521
M98 250809
870446
M97 253248
SUBFAMILY B
SUBFAMILY A

FIG. 19

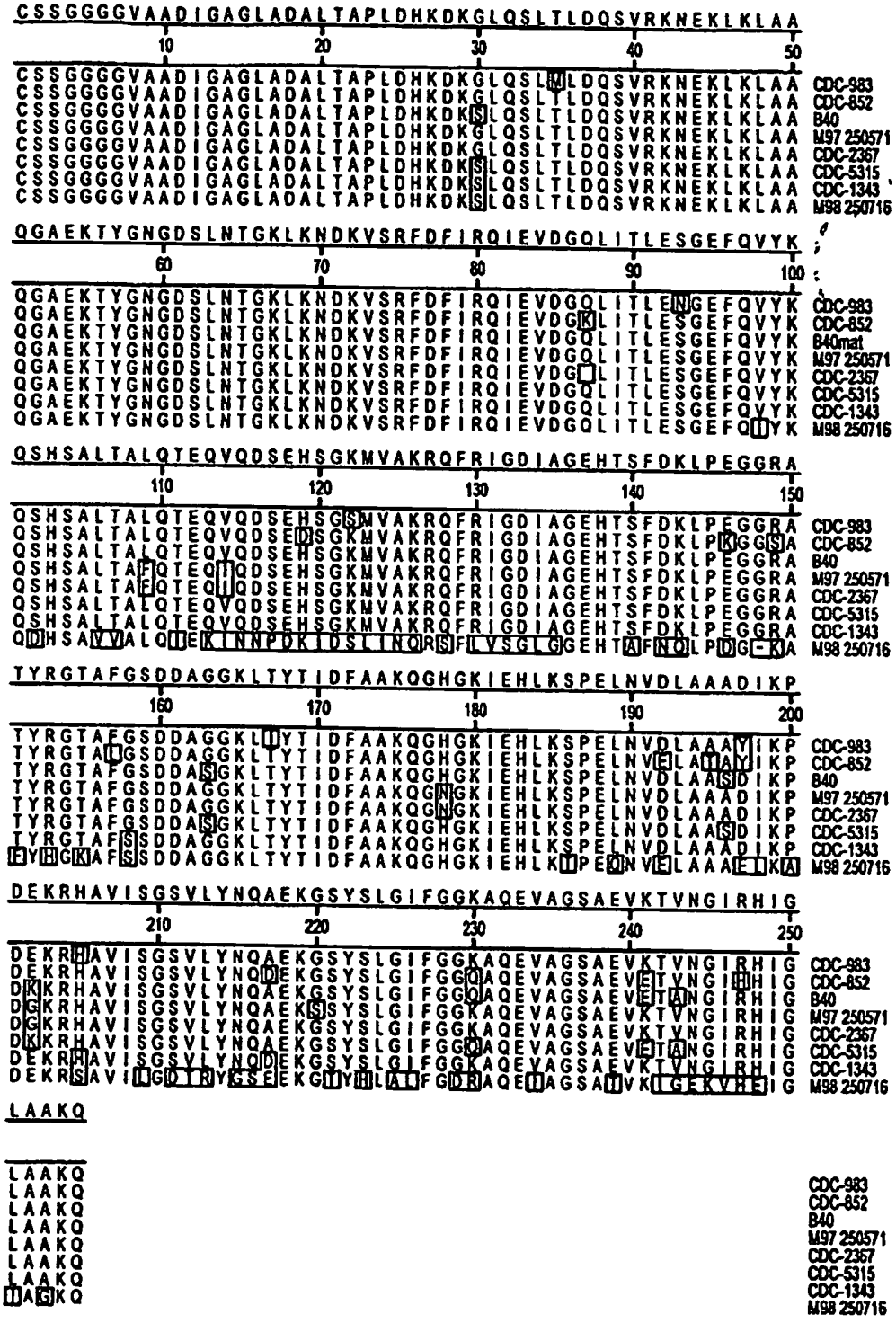

CDC-983
CDC-852
B40
M97 250571
CDC-2367
CDC-5315
CDC-1343
M98 250716
B40mat

FIG. 20

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